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Investigation of Vesicular Rashes for HSV and VZV by PCR

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Vesicular fluid from rashes of 132 patients was tested by a multiplex PCR shown to be specific for herpes simplex virus (HSV) type 1 and 2, and varicella zoster virus (VZV) genomic DNA. The results were compared with those obtained by examination by electron microscopy and virus isolation by cell culture. The PCR did not differentiate between HSV 1 and 2. By PCR, 64 HSV infections and 53 VZV infections were identified, with presumed 100% sensitivity and specificity. Fifteen specimens tested negative by PCR, electron microscopy, and virus isolation for herpes viruses. The sensitivities of virus isolation and electron microscopy for detection of herpes simplex virus were 56% and 80%. For varicella zoster virus, the sensitivities of virus isolation and electron microscopy were 47% and 60%. These data illustrate the advantage of rapid PCR diagnosis of herpes simplex virus and varicella zoster virus in vesicle fluids. ***J. Med. Virol. 54:155–157, 1998.***

KEY WORDS: HSV; VZV; PCR; vesicular rashes; EM

INTRODUCTION

Electron microscopy has an established value for the detection of viruses causing vesicular rashes because it provides a rapid diagnosis. However, fewer instruments are available now but, in our laboratory, electron microscopy is more rapid and sensitive than virus isolation in cell cultures. Herpes viruses are often detected in vesicle fluids in our laboratory. A multiplex polymerase chain reaction [PCR] was evaluated for the rapid diagnosis of herpes simplex virus (HSV) and varicella zoster virus (VZV), and the results were compared with those obtained by electron microscopy and virus isolation.

MATERIALS AND METHODS

Patients

All patients were admitted to Heartlands Hospital with vesicular rashes. Their ages ranged from 4 to 63 years, (mean: 34, median: 42 years, mode: 44 years).

Specimens

Vesicle fluids. Fluid was collected from the vesicles of 132 patients with single or multiple non-genital lesions. The vesicles were punctured using a sterile scalpel or hypodermic needle and the exudate smeared onto a glass microscope slide and allowed to dry. Swabs for attempts at virus isolation were taken of the base of the vesicle and returned to the laboratory in Eagle's minimal essential medium supplemented with 10% foetal calf serum and buffered by 7.5% sodium hydrogen carbonate.

Electron Microscopy

The dried smears of vesicle fluid were reconstituted with 5–10 μl of sterile distilled water. The suspensions were placed onto two electron microscopy grids with carbon strengthened formavar membranes, and left to dry. The grids were washed with three changes of distilled water and stained with 2.5% potassium phosphotungstic acid (pH 6.5). The grids were examined with a Jeol 100CX transmission electron microscope at an accelerating voltage of 80kV.

Virus Isolation in Cultured Cells

Approximately 100 μl of transport medium was used as an inoculum for monolayers of human embryonic lung cells (HELs) in tubes. The monolayers were examined daily for cytopathic effects, for 14 days. Cultures indicating HSV by cytopathic effect were confirmed and typed using an immunofluorescence assay (Diagnostic Products Corp. PathoDx, Los Angeles, CA).

PCR

This was carried out on dried lymph samples stored at 4°C for between 1 and 4 weeks. Strict precautions were taken throughout to avoid contamination [Kwok, 1989;Brown, 1995].

A two-round nested multiplex PCR for HSV types 1 and 2, and VZV was used. The oligonucleotide primers were designed to amplify 141 DNA base-pairs of the gD

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gene of herpes simplex viruses and 208 DNA base-pairs of VZV gene 29 [Davison and Scott, 1986]. In each round four sets of primers were used: PCR

Round 1: 5′ TGCTCCTACAACTC 3′ HSV

5′ CGGTGCTCCAGGATAAA 3′ HSV

5′ ACGGGTCTTGCCGGAGCTGGT 3′ VZV

5′ AATGCCGTGACCACCAAGTATAAT 3′ VZV

PCR Round 2: 5′ ATCCGAACGCAGCCCCGCTG 3′ HSV 5′ TCTCCGTCCAGTCGTTTATCTTC 3′ HSV 5′ ACCTTAAAACTCACTACCAGT 3′ VZV

5′ CTAATCCAAGGCGGGTGCAT 3′ VZV

The PCR reaction conditions were: (first round), 50 mM KCl, 10 mM TRIS, (pH 9 at 25°C), 1.5 mM $MgCl_2$, 200 μM each of dATP, dGTP, dCTP, dTTP, primers at 0.2 μM each, and 1.25 units of Taq DNA polymerase. The second PCR conditions were the same except 1.25mM $MgCl_2$ was used. The optimum Mg^{++}, dNTPs, and oligonucleotide concentrations and oligonucleotide annealing temperatures for both rounds were determined using Oligo®, primer analysis software version 5.0, (MedProbe AS, P.O. 0131 Oslo, Norway). The optimum $MgCl_2$ concentration was confirmed by titration. The samples of lymph were heated to 100°C under mineral oil for 15 minutes and 10 μl was used in the first round PCR giving a final volume of 50 μl. In the second round PCR 1 μl of the product from the first round was added to 50 μl of reaction mixture. In the first round primer annealing was at 52°C for 20 seconds, in the second round 58°C was used. Primer extension was at 72°C for 20 seconds and denaturation at 94°C for 30 seconds. In the first round 30 cycles were used and in the second 35.

The specificity of the PCR was confirmed by sequencing of selected amplicons using the first round PCR 5′ oligonucleotide primer and the ABI automated system.

The sensitivity of the PCR was determined using serial dilutions of infected tissue culture supernatants of predetermined $TCID_{50}$ and by testing serial dilutions of purified HSV genomic DNA, (Sigma, St. Louis, MO) and VZV genomic DNA (Autogen, Autogen Bioclear Butts Farm Devizes, U.K.). All dilutions were prepared in lymphocytic CSF shown to be negative for HSV and VZV DNA by PCR.

RESULTS

The PCR products were visualized after electrophoresis through 2% agarose. VZV gave a PCR product of 208 base-pairs and HSV, 141 base-pairs [Fig. 1]. Ten-fold serial dilutions of CsCl density gradient purified HSV 1 and HSV 2 genomic DNA in lymphocytic CSF was detected down to <1 attogram, (approximately 10–100 genome copies). Similarly, lymphocytic CSF shown to be negative for HSV DNA by PCR was used as a diluent for [Log_{10}] dilutions of 100 $TCID_{50}$ of an HSV 1 isolate. A positive PCR was obtained at the equivalent of 00.0001$TCID_{50}$. The particle-to-infectivity ratio of the virus dilutions was not measured. For the VZV sensitivity the PCR was positive at genomic DNA levels of <1 attogram, (10–100 genome copies) and similar $TCID_{50}$ values were detected.

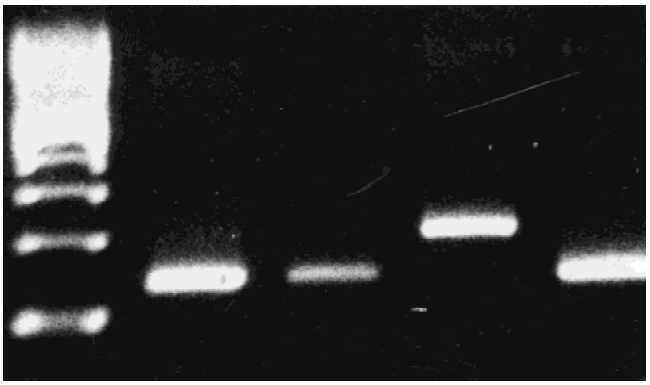

Fig. 1. Two percent agarose gel electrophoresis of the multiplex PCR products from patients and controls. Lane 1: 100 base-pair molecular weight marker, [Gibco-BRL]. Lanes 2 and 3: Clinical specimens, [HSV]. Lane 4: VZV positive control. Lane 5: HSV positive control.

The results of testing 132 samples of vesicle fluid are summarised in Table I. All samples positive by cell culture or electron microscopy were positive by PCR. In addition the use of PCR allowed the detection of virus in a further 21% of cases (HSV 12.9% and VZV 8.3%). Cell culture failed to detect 43% of HSV cases and 57% of VZV cases. Electron microscopy failed to detect 24% of cases, but cannot differentiate HSV and VZV. Assuming 100% sensitivity for PCR, for herpes simplex EM achieved 80% sensitivity and cell culture 56% sensitivity. Similarly for varicella zoster EM achieved 60% and cell culture 43%.

Nucleotide base sequence analysis of HSV and VZV derived PCR products showed 99% homology with 10 sequences each obtained from GenBank [data not shown]. Mixtures of VZV and HSV from 1:10 to 10:1, respectively, were positive for both viruses by PCR. However, mixed infections were not seen in the clinical samples.

DISCUSSION

The results showed that all clinical specimens positive by cell culture or electron microscopy were also positive by PCR, but that PCR detected a further 21% of samples indicating its superior sensitivity. Clinical details gave some support to the positive PCR diagnosis in those cases negative by electron microscopy and cell culture (Table II). Forty-eight percent of the cases were diagnosed as HSV infections, in contrast to the 40% diagnosed as VZV.

The PCR detected successfully HSV and VZV genomic DNA on dried lymph samples stored for up to 4 weeks. The results from the stored samples does demonstrate the robustness of the PCR, but this technique is designed for real-time testing.

Electron microscopy can detect many viruses. It has the advantage of speed for diagnosis of vesicular rashes and this is especially important for atypical rashes, commonly observed amongst immunocompromised pa-

TABLE I. The Results of Testing 132 Samples of Vesicle Fluid by PCR, Virus Isolation, and EM

A] HSV PCR Positive

Result	Total	Percentage
EM, T/C Positive*	36	27.3
EM, Positive T/C Negative	11	8.3
EM, T/C Negative	17	12.9
Total	64	48.5

[*24 Type 1 (66.6%), 12 Type 2 (33.3%)

B] VZV PCR Positive

Result	Total	Percentage
EM, T/C Positive	23	17.4
EM Positive, T/C Negative	19	14.4
EM, T/C Negative	11	8.3
Total	53	40.1

C] PCR Negatives

EM, T/C** Negative	15	11.4
	132	[100]

[**Includes 2 Molluscum contagiosum and 1 Orf virus]

TABLE II. Patient Clinical Details vs. Positive PCR Results

	PCR Result		
Clinical details	HSV	VZV	Total
? VZV	21	18	39
? HSV	26	0	26
Rash	10	35	45
Vesicles	7	0	7

tients [Dlugosch et al., 1991; Koropchak et al., 1991]. In such cases rapid antiviral chemotherapy is essential. However, it cannot distinguish between HSV and VZV.

Low isolation rates for VZV in cultured cells have been described previously [Dlugosch et al., 1991], who reported a rate of 23% and stressed the importance of taking the sample of vesicle fluid within 5 days of onset of symptoms and inoculating cell cultures on the same day. Their findings were confirmed in our study.

The difference in the detection rates of electron microscopy and virus isolation compared to PCR, were highly significant ($P < 0.001$; Student's *t*-test). We now demonstrate the limited sensitivities of both methods, (especially isolation in cell cultures), which calls into question their future use for the diagnosis of vesicular rashes.

Although PCR remains a 24 hour assay, (compared to the potential 1 hour EM test), PCR detects 24% more positive fluids than EM. These results demonstrate clearly the usefulness of PCR as an alternative to electron microscopy and virus isolation. Furthermore, it is a more rapid and sensitive test. A convincing argument for PCR as the first-line diagnostic test for viral infections of the central nervous system has been made [Jeffrey et al., 1997]. Similarly, this study has also shown the value of PCR compared to traditional methods of virus detection.

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REFERENCES

Brown DWG (1995): The current role of PCR in diagnostic and public health virology. In Clewley JP (ed): "The Polymerase Chain Reaction (PCR) for Human Viral Diagnosis." London, CRC Press, pp 13–21.

Davison AI, Scott JE (1986): The complete DNA sequence of varicella zoster virus. Journal of General Virology 67:1759–1777.

Dlugosch D, Eis-Hübinger, Kleim J-P, Kaiser R, Bierhoff E, Schneweis KE (1991): Diagnosis of acute and latent varicella-zoster virus infections using the polymerase chain reaction. Journal of Medical Virology 35:136–141.

Jeffrey KJM, Read SJ, Peto TEA, Mayon-White RT, Bangham RM (1997): Diagnosis of viral investigations of the central nervous system: clinical interpretation of PCR results. Lancet 349:313–317.

Koropchak C, Graham G, Palmer J, Winsber M, Ting SF, Wallace M, Prober CG, Arvin AM [1991]: Investigation of varicella-zoster virus infection by polymerase chain reaction in the immunocompetent host with acute varicella. Journal of Infectious Diseases 163: 1016.

Kwok S, Higuchi R (1989): Avoiding false positives with PCR. Nature 339:237–238.